Theligonum cynocrambe: Developmental morphology of a peculiar rubiaceous herb

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Theligonum cynocrambe: developmental morphology of a peculiar rubiaceous herb

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Key words: *Rubiaceae*, *Rubioideae*, *Theligonum cynocrambe*. – Developmental morphology, life history, flower structure, systematics.

Abstract: The annual Mediterranean herb *Theligonum cynocrambe* shows a peculiar combination of morphological characters, e.g., switch from decussate to spiral phyllotaxis with 90–100° divergence, combined with a change from interpetiolar to lateral stipules, anemophily, lack of calyx, flowers often dimerous to trimerous, corolla fused in both male and female flowers, male flowers extra-axillary, with 2–19 stamens per flower, female flowers axillary, with inferior uniovulate ovary, basilateral style and perianth, nut-like fruits with elaiosome. In male flowers the androecium emerges as an (uneven) elliptical rim with a central depression. This common girdling primordium is divided up into several stamen primordia. In male flowers with low stamen number the stamen primordia may occupy the corners alternating with the corolla lobes. There are no epipetalous androecial primordia that secondarily divide into stamens. Male flowers occasionally show a hemispherical base that may be interpreted as remnant of the inferior ovary. In female flowers a ring primordium grows into a tube on which the petal lobes arise. The perianth and style become displaced adaxially by uneven growth of the inferior ovary. The ovary is basically bilocular. The lower region of the ovary is provided with a septum that is overtopped and hidden by the single curved ovule. *Theligonum* is referred to the *Rubiaceae-Rubioideae*, with the *Anthospermeae* and *Paederieae* as most closely related tribes.

Theligonum cynocrambe is an annual prostrate herb with shoots of 5-20(-75) cm length, with cordate and petiolate foliage leaves (up to 2.5 cm long), and inconspicuous flowers arising from the same nodes as the foliage leaves (Fig. 1). *Theligonum cynocrambe* occurs in the Mediterranean region (from Syria to Portugal and Morocco) and on the Canary Islands. It is often found growing on damp or shady soils, rocks and walls (ULBRICH 1933, 1934). The genus *Theligonum* L. consists of three species that show a disjunct geographic distribution in the Northern hemisphere: one Mediterranean species (*T. cynocrambe* L.) and two Asiatic species. *Theligonum japonicum* OKUBO & MAKINO (from Central and S Japan) seems to be perennial and monoecious (YAMAZAKI 1993). *Theligonum macranthum* FRANCH. (from W China, former Tibet) is annual and supposedly dioecious (ULBRICH 1933, 1934; PUFF 1986: 520).

Theligonum is now regarded as a member of the *Rubiaceae* (WUNDERLICH 1971; ROBBRECHT 1988, 1993; THORNE 1992; NICHOLAS & BAIJNATH 1994; BREMER & al. 1995; NATALI & al. 1995; BREMER 1996; MANEN & NATALI 1996) or as a closely related family within the *Gentianales* (DAHLGREN 1989) or *Rubiales* (CRONQUIST 1981). Features in common with *Asteridae* – and unlike the *Caryophyllidae* – are, e.g., the tubular perianth, S-sieve tube plastids, iridoid compounds, seeds with endosperm, and the unitegmic tenuinucellate ovules (WUNDERLICH 1971, CRONQUIST & THORNE in BEHNKE & MABRY 1994: 5–25).

In order to stress the homology with the corolla of other *Rubiaceae*, the perianth of *Theligonum* flowers (both sexes) is interpreted as a corolla (with fused petals). This view is based on the assumption that the calyx is totally suppressed. Such an assumption seems to be justified because calyx reduction as an evolutionary trend is also observable in other *Rubiaceae* (RUTISHAUSER 1985, PUFF 1986, ROBBRECHT 1988: 74). Nonetheless, the neutral term "perianth" is preferred here, as it avoids circular reasoning.

There is already a considerable amount of valuable information on *Theligonum*. Some developmental and morphological data, however, are still lacking or have been misinterpreted, especially with respect to the primordial stages of foliage leaves, stipules, male and female flowers, and micromorphological data. Since BALICKA-IWANOWSKA (1897), SCHNEIDER (1914), ULBRICH (1934), WUNDERLICH (1971) and ROBBRECHT (1988) it is believed that the male flowers show secondarily increased stamen number due to the presence of two or three common epipetalous primordia that divide into 6–30 stamens. Such a dédoublement of the androecium in *Theligonum* would be exceptional for the *Rubiaceae* and most other *Asteridae* (ROBBRECHT 1988: 85). Other exceptions in the subclass would be *Coprosma nephelephila* within the *Rubiaceae* (FLORENCE 1986), the *Hoplestigmataceae* (RONSE DECRAENE & SMETS 1995) and possibly the *Dialypetalanthaceae* (ROBBRECHT 1993, PIESSCHAERT 1995). Another hypothesis considers the male flowers as pseudanthia consisting of naked flowers with about four stamens each (WUNDERLICH 1971). Pistillate flowers are interpreted as unicarpellate by most authors. However, Wunderlich postulates a derivation from two carpels, linking *Theligonum* with the basically bicarpellate *Rubiaceae*. These statements will be tested in this paper.

Materials and methods

Material was collected either in the wild (Sardinia, collections 1982/1995) by the first author, or grown from seed at the Botanical Institutes of Leuven and Zürich. Seed was obtained from the wild (Sardinia) as well as from the Botanic Gardens of Göttingen, Glasgow and Marburg. Plants from these three gardens did not differ morphologically. Whole plants were fixed in formalin - acetic acid - ethyl alcohol (FAA) and later stored in 70% ethanol. Voucher specimens (herbarium and pickled) are kept at the Botanical Institutes of Leuven (LV) and Zürich (Z). For the scanning electron microscopy the dissected shoot tips were dehydrated in acetone or dimethoxymethane, then critical-point dried with carbon dioxide and sputter-coated (Au). The micrographs were taken with a Cambridge S4 (Z) and a Jeol JSM 6400 (LV) scanning electron microscope at 20 kV.

For light microscopy the material was run through an alcohol and alcohol-tertiary butanol series and was next embedded in paraffin. Serial sections, 10 µm thick, were stained with safranin and fast green.

Results

Foliage leaves: from decussate to 1/4 spiral phyllotaxis. There is a remarkable switch from decussate to spiral phyllotaxis that is not common in other genera of flowering plants. Cotyledons and seedling leaves show decussate phyllotaxis with membraneous interpetiolar stipules (Figs. 3–4). The cotyledons do not differ morphologically from the leaves; they also bear shoot buds and colleters in their axils. There are (2-)3–4(-6) leaf pairs (including the cotyledons) prior to the switch to the helical (alternate) phyllotaxis of the adult plant. Each vegetative axillary shoot starts with a few leaf pairs before switching to an alternate phyllotaxis. Vegetative axillary shoots in addition to pistillate flowers are confined to the lower part of the main stem (nodes 1-3). Higher up only pistillate flowers are produced in the leaf axils.

The spiral phyllotaxis of the adult plant is unique among angiosperms. The foliage leaves (each with two lateral stipules) are arranged along a helix with c. 90–100° divergence (Figs. 1, 2, 51–53; LOISEAU 1969). This type is called 1/4 spiral phyllotaxis because the divergence is close to 90° (ULBRICH 1933, 1934). This phyllotaxis type may be seen as a case of extreme anisophylly, with the (+) leaves of the basically decussate pattern arranged along a helix, whereas all (−) leaves are nearly or completely suppressed (IRMISCH 1856; ROBBRECHT 1988, 1993). Also the stipules at each node are unequal, at least during their development, with the anodic stipule (Sa)[1] favoured as compared to the cathodic one (Sc2 in Figs. 6–8, 11, 51–53, see LOISEAU 1969). The anodic stipule (e.g., Sa4 in Fig. 51) of a leaf is adjacent to the next younger leaf (3) whereas the cathodic stipule (Sc4) is closer to the next older leaf (5 in Fig. 51). *Theligonum* shoots show a slight anisophylly already in the lowermost decussate zones (e.g., Figs. 2, 5). Younger stages of the development of the shoot show stipules which are initially larger than the associated leaves (Figs. 5–7). Stipules may bear an apical gland or colleter. The switch to the 1/4 spiral arrangement is not as gradual as described by PUFF (1986: 519). Only in a few nodes of the transition zone rudiments of the normally suppressed (−) leaf may be observed (IRMISCH 1856, EICHLER 1878, SCHNEIDER 1914). We occasionally observed a short scale in the position of the (−) leaf.

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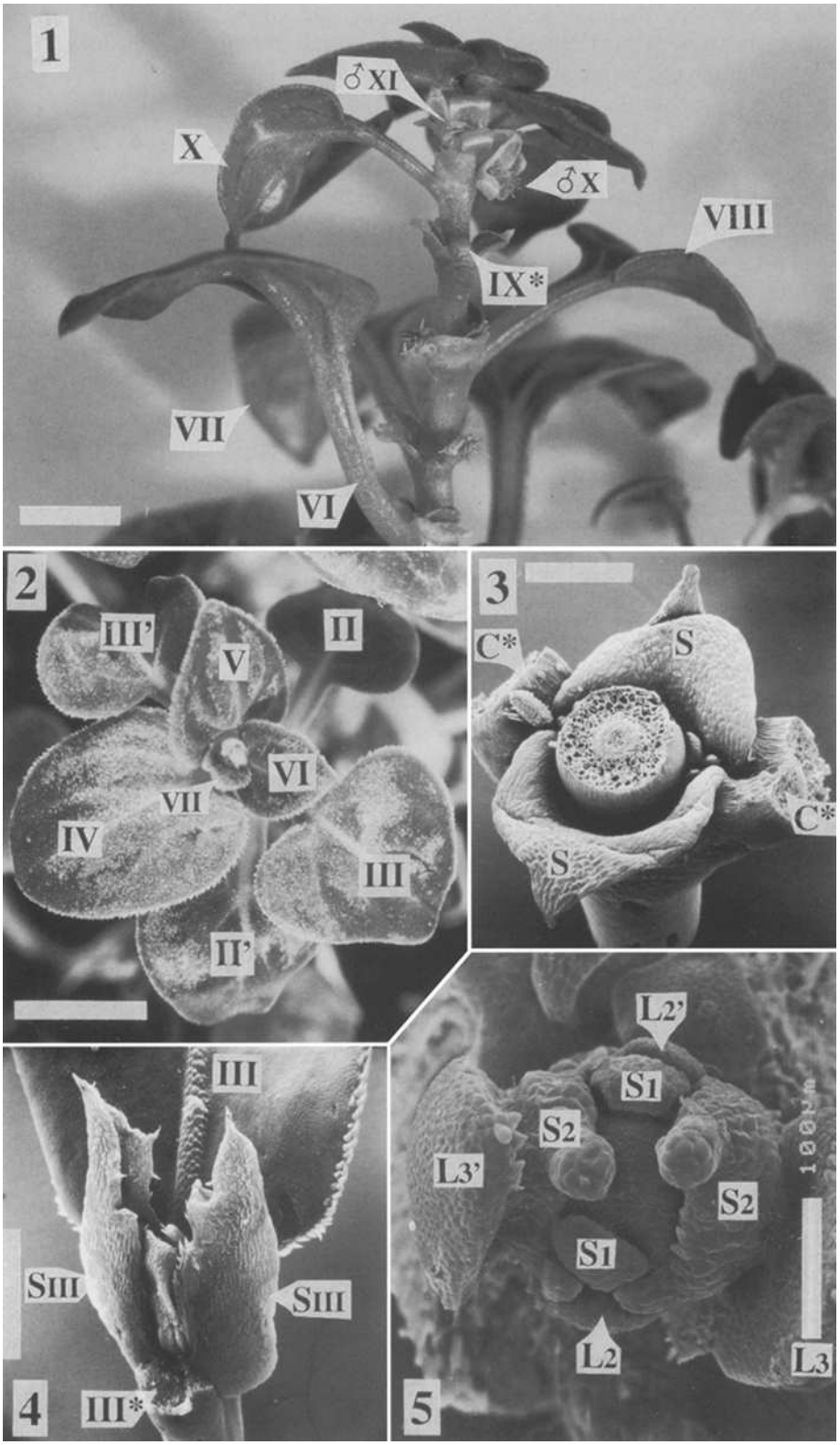
[1] Explanation of the terms anodic and cathodic: The terms anodic and cathodic are used in shoots with a spiral phyllotaxis and asymmetric leaf sheaths and/or unequal lateral stipules. The anodic stipule points towards the distal end of the ontogenetic helix whereas the cathodic stipule points towards the proximal end (LOISEAU 1969, KIRCHOFF & RUTISHAUSER 1990). In *Theligonum* with spiral phyllotaxis and two male flowers opposite the leaves, these two flowers (e.g., ♂a4 and ♂c4 in Fig. 51) are also positionally defined with respect to the direction of the ontogenetic helix. The anodic flower (e.g., ♂a4) is closer to the anodic stipule (Sa4) which is covering the next younger leaf (3 in Fig. 51). The cathodic flower (e.g., ♂c4) is closer to the cathodic stipule (Sc4) that is in front of the next older leaf (5 in Fig. 51).


1
♂XI
X
♂X
VIII
IX*
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VI
2
III'
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III
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SIII
III*
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L2'
S1
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S1
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L3

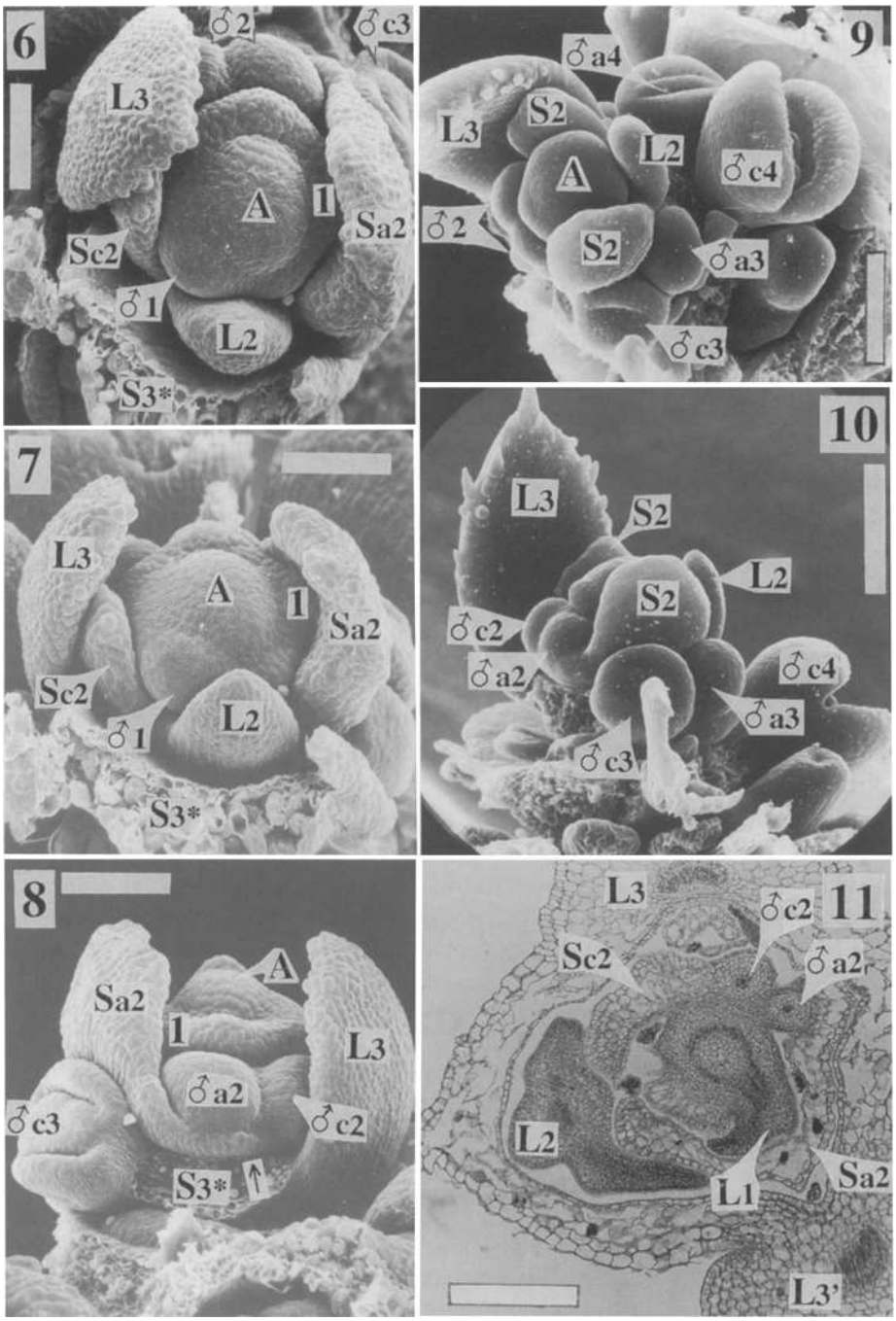
Higher up the (−) leaf subtending the male flowers is lacking except for an inconspicuous bump or appendage that may or may not be present in early developmental stages (Fig. 8: arrow).

Monoecy, anthesis of male and female flowers, pollen structure. Instead of the bisexual flowers that are typical for most *Rubiaceae* there is a differentiation into female and male flowers within each plant (monoecy) and even within each node of the adult shoot zone. Female flowers arise in groups of (1-)2–4(-7) in the axils of foliage leaves, and are already present in the axils of the seedling leaves. Male flowers only occur higher up after the change to single leaf nodes (Figs. 1, 51; LOISEAU 1969).

At anthesis the length of the female flower (measured from ovary base to stigma) is 0.8–2 mm (Figs. 39, 40). The cylindrical stigma (with papillate cells) protrudes beyond the corolla tube for a length of about 200–300 µm (Figs. 39–41). As compared to the male flowers of the same node, development of the female flowers often seems to follow that of the male flowers. Male flowers most often arise in pairs, rarely in groups of three. They are extra-axillary and opposite to the spirally arranged foliage leaves (Figs. 1, 8–10, 31, 51). Such a flower position seems advantageous for wind pollination, and is combined with pendulous stamens, elongate threadlike filaments, and powdery pollen. The length of male flowers during anthesis is c. 3–5 mm. In this stage the corolla lobes are coiled backwards (Figs. 30–31). We observed a high number of stomata in transversal rows, and the process of coiling is probably related to changes in the water content of the cells. The dorsifix anthers are elongate and dehisce by two longitudinal slits (one per theca). Afterwards the empty anthers may or may not become helically twisted during drying (Figs. 30–31).

In the material studied we only found 6- to 7-zonoporate pollen with a reticulate exine provided with muri covered with numerous suprategillar elements (Figs. 32–33). (3-)4–8 equatorial apertures (pori) per pollen were described in *Theligonum* spp. by BALICKA-IWANOWSKA (1897), ULBRICH (1934), PRAGLOWSKI (1973), NOWICKE & SKVARLA (1979), and ROBBRECHT (1988).

Figs. 1–5. *Theligonum cynocrambe*. – Fig. 1. Lateral view of an adult erect shoot, showing the stalked foliage leaves VI-XI with spiral phyllotaxis along elongated axis (IX*, with blade removed). More distal leaves crowded in terminal rosette. The leaf bases with lateral stipules. The male flowers (♂X, ♂XI) opposite the leaves X-XI are in anthesis (with glossy coiled corolla lobes). – Fig. 2. Top view of the terminal rosette of another shoot after having switched from decussate (II-II′/III-III′) to spiral phyllotaxis (IV–VII). The foliage leaves are numbered according to their ontogenetical order. The spiral phyllotaxis shows divergence angles of c. 100°. – Fig. 3. Cotyledonary node of seedling, with basal portions of the two cotyledons (C*, removed) and two membranous stipules (S) in intercotyledonary position. Note the colleter(s) in the axils of the cotyledons. – Fig. 4. Terminal bud of seedling axis, with third pair of foliage leaves (III/III*, one blade removed). Note the two interpetiolar stipules (SIII) that are folded along the median plane covering the terminal bud. – Fig. 5. Top view of young vegetative shoot, with decussate arrangement of leaves. Note the unequal size of leaf pair L3/L3′. The interpetiolar stipules (S2) of the next younger node are exceeding the size of the leaf primordia L2/L2′. S1 are stipules of the youngest node (leaf primordia hidden). – Bars: Figs. 1–2, 5 mm; Figs. 3–4, 1 mm; Fig. 5, 100 µm



Figs. 6–11. *Theligonum cynocrambe*. Adult shoot tips, after switch to 1/4 spiral phyllotaxis. – Figs. 6–8. Three views of adult shoot apex, ontogenetical helix in counterclockwise direction. Youngest node next to shoot apical meristem (A) with girdling primordium (1) not clearly differentiated into leaf bulge and associated stipular primordia. ♂1, initial male flower belonging to node 1. Next older node with leaf primordium (L2), associated unequal stipules: Sa2 (anodic stipule) larger than Sc2 (cathodic stipule). From the next older leaf (L3) both stipules removed (S3*). ♂a2/♂c2, male flower primordia of

Development and position of male flowers. Male flowers occupy the site of the suppressed (−) leaves. In early developmental stages we occasionally detected a rudiment of the subtending (−) leaf (Fig. 8: arrow). There are mostly two (rarely three) male flowers of slightly unequal size and shape per node. The cathodic flower primordium (e.g., ♂c4 in Fig. 9) is favored whereas the anodic flower primordium (e.g., ♂a4) is slightly retarded in development and anthesis (BALICKA-IWANOWSKA 1897). However, the cathodic flower often ends up as being the smaller flower of the pair, showing fewer stamens than the anodic one (Figs. 26, 27). Moreover, as compared to the anodic flower (laterally flattened and with two corolla lobes) the cathodic one is normally less flattened and quite often provided with three corolla lobes instead of two (Figs. 17, 18). Rudimentary scales associated with the male flower groups are occasionally present (Figs. 8, 17; EICHLER 1878, ULBRICH 1934). Thus, it is possible to interpret the developmental sequence of two (or rarely three) male flowers per site as a highly reduced cyme, with nearly or completely lacking prophylls, analogous to the female flower groups (see below).

Perianth development in male flowers. Male flowers are initiated as hemispherical bumps (Figs. 8, 10). Then they may produce a circular rim (Figs. 12–14), or they become flattened with two (or three) perianth primordia arising on the periphery (Figs. 15, 16). In dimerous flowers the perianth arises in the form of two flat primordia (one adaxial and one abaxial). In trimerous flowers there arise three flat primordia (one abaxial and two adaxial). They tend to grow at unequal rates. There is always one perianth primordium facing the abaxial side (Figs. 17, 18). The adaxial lobe is usually larger and may enclose more stamens in a row than the abaxial lobe (Figs. 23, 24). Before anthesis a basal tube is produced by intercalary growth below the perianth lobes (Figs. 28, 30, 31). The two (or three) perianth lobes show valvate aestivation before anthesis (Figs. 28, 29). When the flowers open, the lobes are reflexed, exposing the long filaments and anthers (Figs. 30, 31).

Meristic variation and stamen initiation in male flowers. Unlike most members of the *Rubiaceae*, *Theligonum cynocrambe* shows a considerable meristic variation, especially with respect to the male flowers. Such a situation may be called "leakiness" of the *Rubiaceae*-"Bauplan" (as proposed by V. ALBERT, pers.

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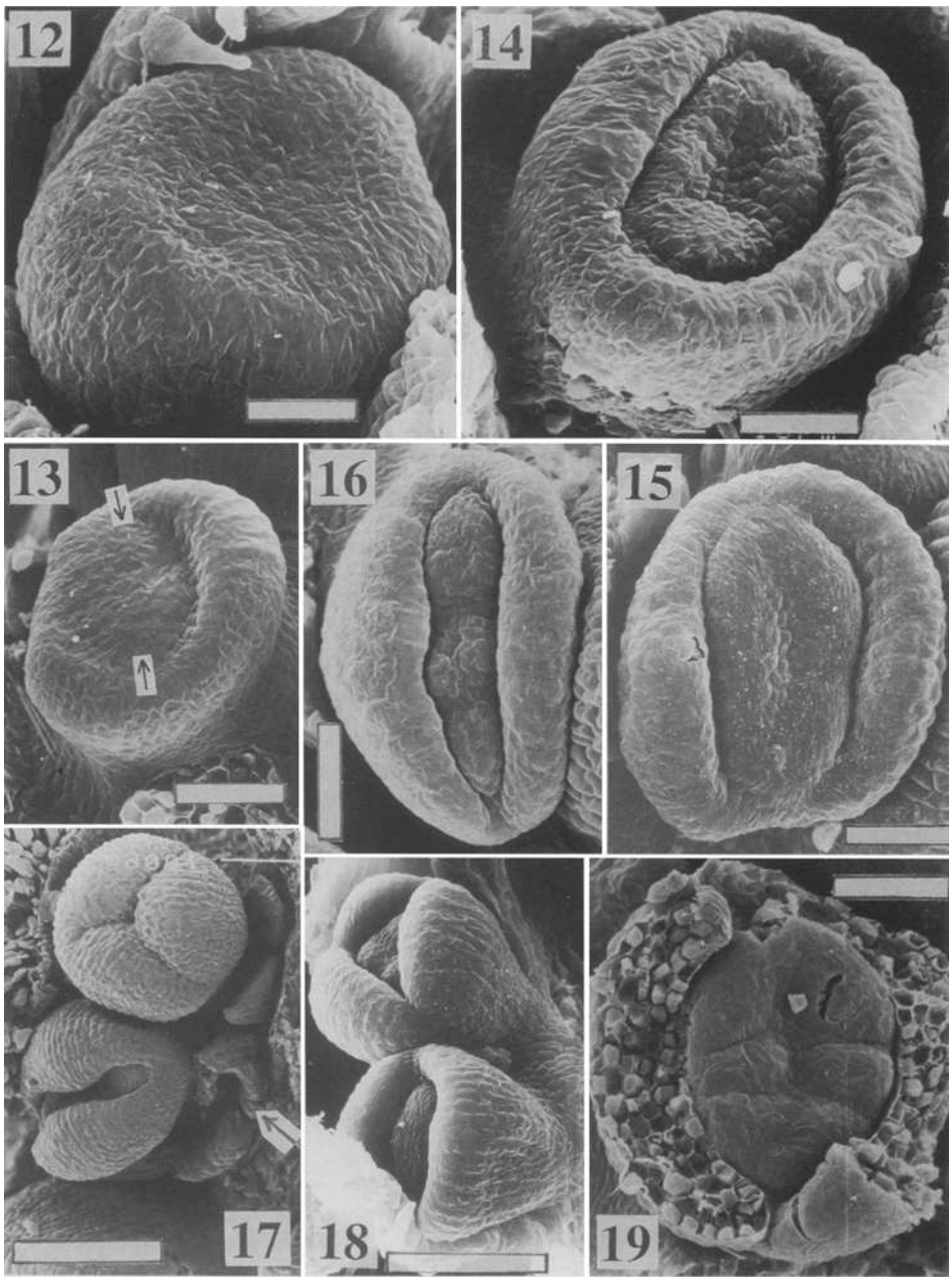
node 2 (a = anodic flower, c = cathodic one); ♂c3, primordium of cathodic male flower of node 3. Black arrow in Fig. 8 points to primordial bump that may be interpreted as rudimentary leaf subtending the male flowers. – Figs. 9–10. Two views of another shoot apex, with clockwise helix. Shoot apical meristem (A) with initial stage of new appendage node (not labeled). Second appendage node with leaf primordium (L2), two stipules (S2), two male flower primordia (♂2) opposite to the leaf. Leaf (L3) and male flower pair (♂a3,♂c3) opposite each other. Stipules of older nodes 3–4 removed. ♂a4,♂c4, anodic and cathodic male flowers of fourth node with unequal size. – Fig. 11. Transverse section of the shoot apex at the switch from decussate to spiral phyllotaxis with leaf pair (L3/L3′). Ontogenetical helix of younger leaves in counter-clockwise direction: L2 with anodic (Sa2) stipule broader than cathodic (Sc2) one. L1, insertion area of even younger leaf. ♂a2,♂c2, anodic and cathodic male flowers opposite to L2. – Bars: Figs. 6–8, 100 μm; Figs. 9–10, 200 μm; Fig. 11, 300 μm



Figs. 12–19. *Theligonum cynocrambe*. Early development of male flowers. – Fig. 12. Early stage of perianth formation of cathodic flower. Note depression surrounded by a continuous rim. – Fig. 13. Anodic flower just after perianth initiation. Arrows point to two alternipetalous androecial primordia. – Fig. 14. Young flower with girdling perianth, and an elliptical rim of four (?) androecial primordia surrounding a central depression. – Fig. 15. Young cathodic flower with two corolla lobes and an elliptical androecial rim surrounding a central depression. Stamen primordia on rim not yet observable. – Fig. 16. Young flower with three androecial primordia parallel to the two corolla lobes. – Fig. 17. Two flowers with their perianth. Cathodic flower (above) trimerous and slightly preceding anodic

comm.). In the material studied the stamen number varies from two to six in smaller flowers (Figs. 22, 25, 27) to up to 11–19 in larger flowers (Figs. 24, 26). In male flowers there is normally no rudiment of the styles and the inferior ovary. Occasionally male flowers show a nearly globular base at anthesis that may be interpreted as remnant of the inferior ovary (Fig. 30). Cross-sections through such swollen floral bases do not add more information to corroborate the hypothesis on the rudimentary inferior ovary in male flowers (A. IGERSHEIM, unpubl.).

Stamen initiation patterns are quite variable. Depending on the number of perianth lobes (two or three) of the male flowers, stamen initiation starts with the formation of two or three alternipetalous bumps (arrows in Fig. 13, also Figs. 16, 18). When these primordia become directly stamens, then there are only two or three stamens per flower. In other floral primordia with two perianth lobes, however, the androecium emerges as an elliptical rim (common girdling primordium) with a central depression (Figs. 14, 15). Then, this rim is nearly simultaneously divided up into five to eleven or even more stamen primordia. Often one stamen ends a row in alternipetalous position (Figs. 23, 24). The development of the median stamens (in front of the corolla lobes) is normally not accelerated as compared to the lateral ones (Figs. 16, 19). In older flowers with a higher stamen number, the middle stamens of a row may become displaced to the center of the flower or more externally (Figs. 24, 26). Not obvious in our material is the fascicle (cluster) formation among the stamens. Only rarely two filaments were fused at their base (Fig. 27: arrow). It was not possible for us to see fascicles of mainly two, four, or six stamens as described by BALICKA-IWANOWSKA (1897), SCHNEIDER (1914) and WUNDERLICH (1971: 370). Moreover, male flower primordia do not show bulge-shaped epipetalous common primordia prior to stamen initiation. Such common primordia (two or three according to the tepal number) were described and illustrated by BALICKA-IWANOWSKA (1897: figs. 6, 7) and SCHNEIDER (1914: fig. 9). CARUEL'S (1873) observations tend to be more correct, as he described the initiation of two outer alternipetalous primordia, followed by the epipetalous stamens in two rows. However, we often observed a continuous rim, i.e., a common girdling primordium around a central depression, though the extremities at the perianth interstices may not always be clearly developed (Figs. 14, 15, 19, 23). Additional material needs to be studied in order to estimate the whole range of stamen initiation patterns in *Theligonum*!

Development and position of female flowers. In the material studied there are (1-)2–3(-5) female flowers per leaf axil. The set of female flowers of a leaf axil is called "female unit" in the present paper. According to BALICKA-IWANOWSKA (1897) and WUNDERLICH (1971) there are normally three flowers (fruits) per female unit, occasionally even up to five flowers (fruits) on vigorous shoots. Each female unit is best interpreted as a cymose inflorescence, because there is a clear

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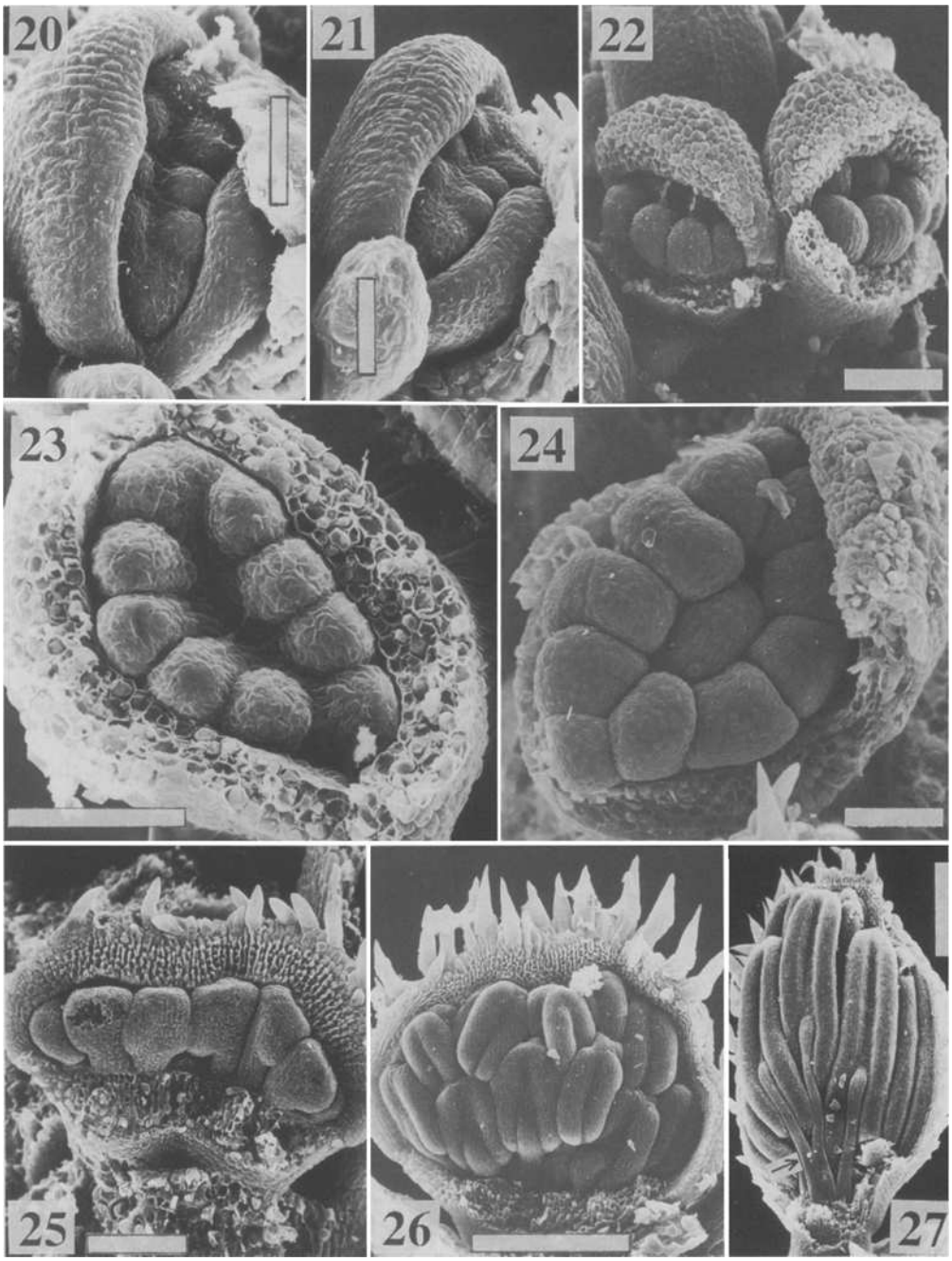
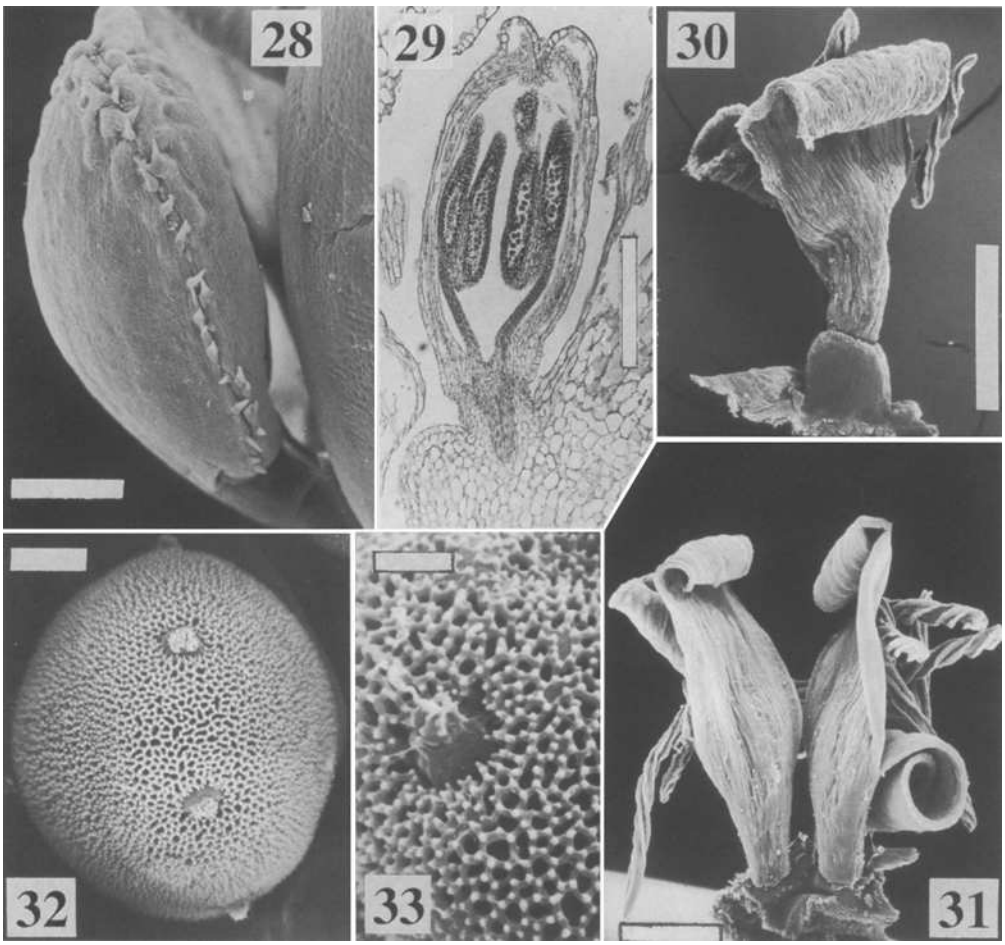
dimerous flower (below). Arrow points to scales that may belong to the rudimentary leaf supporting the male flowers. – Fig. 18. Younger flower pair. Again cathodic flower (above) trimerous and anodic flower dimerous (below). – Fig. 19. Young anodic flower with androecial primordia differentiating on the rim (the two perianth lobes removed). – Bars: Figs. 12–16, 19, 30 μm; Figs. 17, 18, 100 μm



Figs. 20–27. *Theligonum cynocrambe*. Further development of male flowers. – Figs. 20, 21. Two views of anodic flower after inception of eight stamen primordia. – Fig. 22. Two flowers after removal of the abaxial perianth lobe. The cathodic flower (on the right) with five young stamens whereas the anodic flower with at least six stamens. – Fig. 23. Apical view of flower with nine stamen primordia along two rows inside the two perianth lobes (removed). The adaxial lobe (on the left) is curved and encloses more stamens than the abaxial lobe. – Fig. 24. Slightly older flower bud with 12 stamen primordia, including a central stamen that may be displaced by crowding. Note one stamen at both ends. – Fig. 25.



Figs. 28–33. *Theligonum cynocrambe*. Male flowers prior and at anthesis, pollen structure. – Fig. 28. Lateral view of nearly mature flower bud. Note the valvate aestivation of the perianth lobes fringed with unicellular hairs. Perianth tube still very short. – Fig. 29. Longisection through anodic flower bud at a developmental stage similar to Fig. 28. Note the central depression and the slight adnation of the stamens to the perianth base. – Fig. 30. Flower during anthesis, with anthers already dehisced and the corolla lobes coiled backwards. Note the globular floral base that might be a rudimentary inferior ovary. – Fig. 31. Two flowers at anthesis, seen from adaxial side, with less prominent floral bases. Both flowers with two coiled corolla lobes. Note the helical twisting of the dehisced anthers. – Fig. 32. Hexazonoporate pollen, equatorial view. – Fig. 33. Close-up of pollen, with porate aperture, reticulate exine and warts. – Bars: Figs. 28, 29, 300 μm; Figs. 30–31, 1 mm; Fig. 32, 5 μm; Fig. 33, 2 μm

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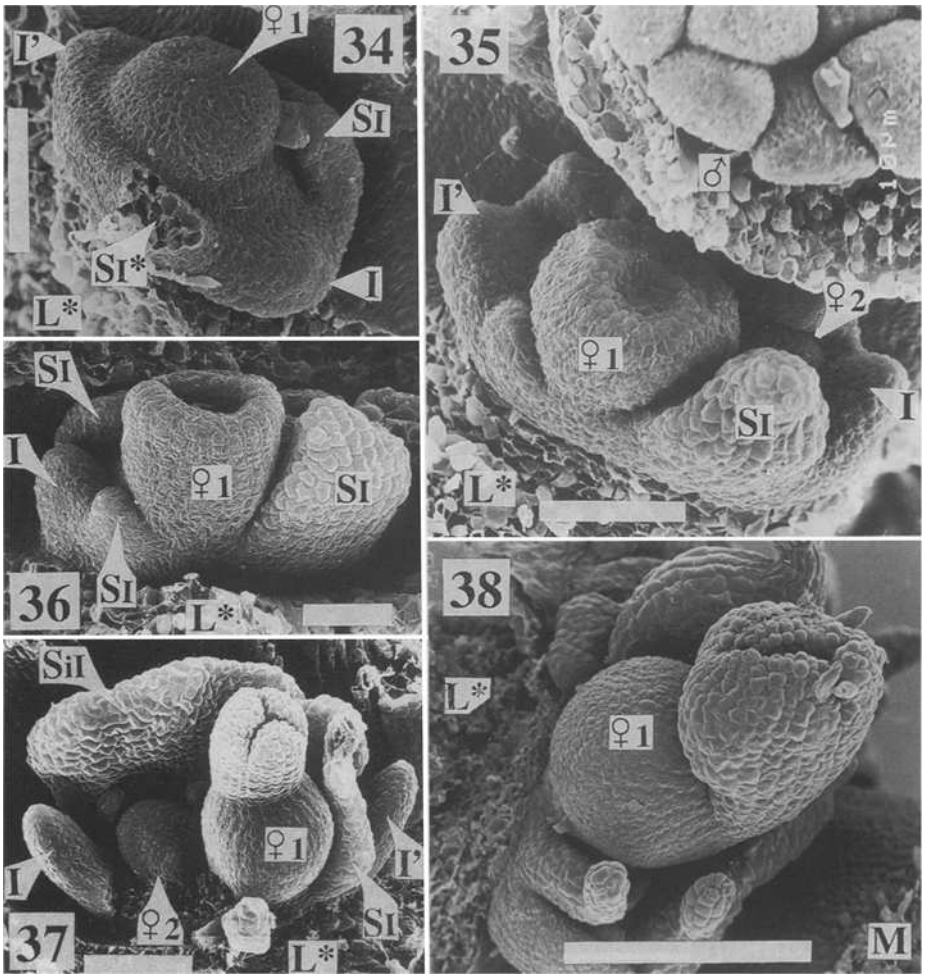
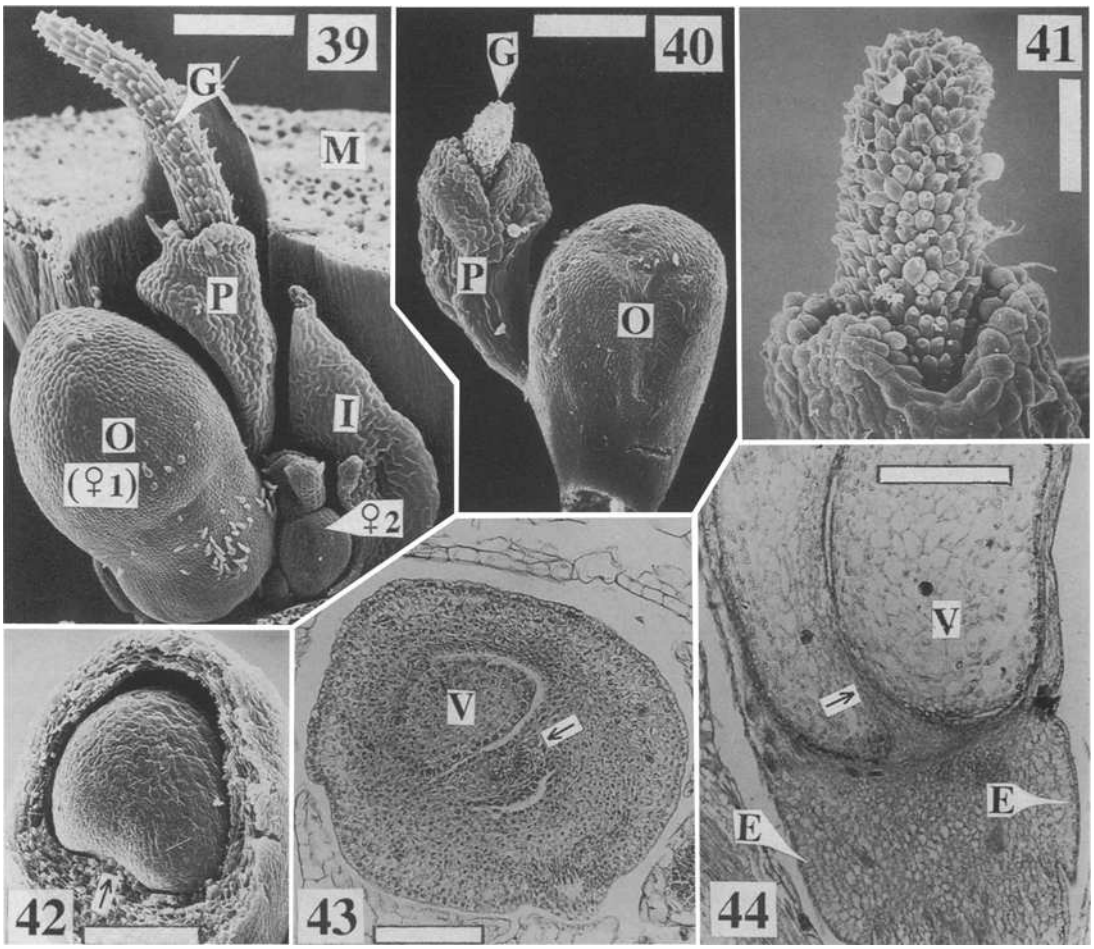
Older flower bud with six stamens along one row. – Fig. 26. Anodic flower, one tepal removed, 11 stamens observable. – Fig. 27. Cathodic flower (sister flower of anodic flower shown in Fig. 26), perianth partially removed. The six stamens much further developed than those of sister flower. Two filaments are fused at base (arrow). – Bars: Figs. 20, 21, 23, 24, 50 μm; Figs. 22, 25, 100 μm; Figs. 26, 27, 300 μm

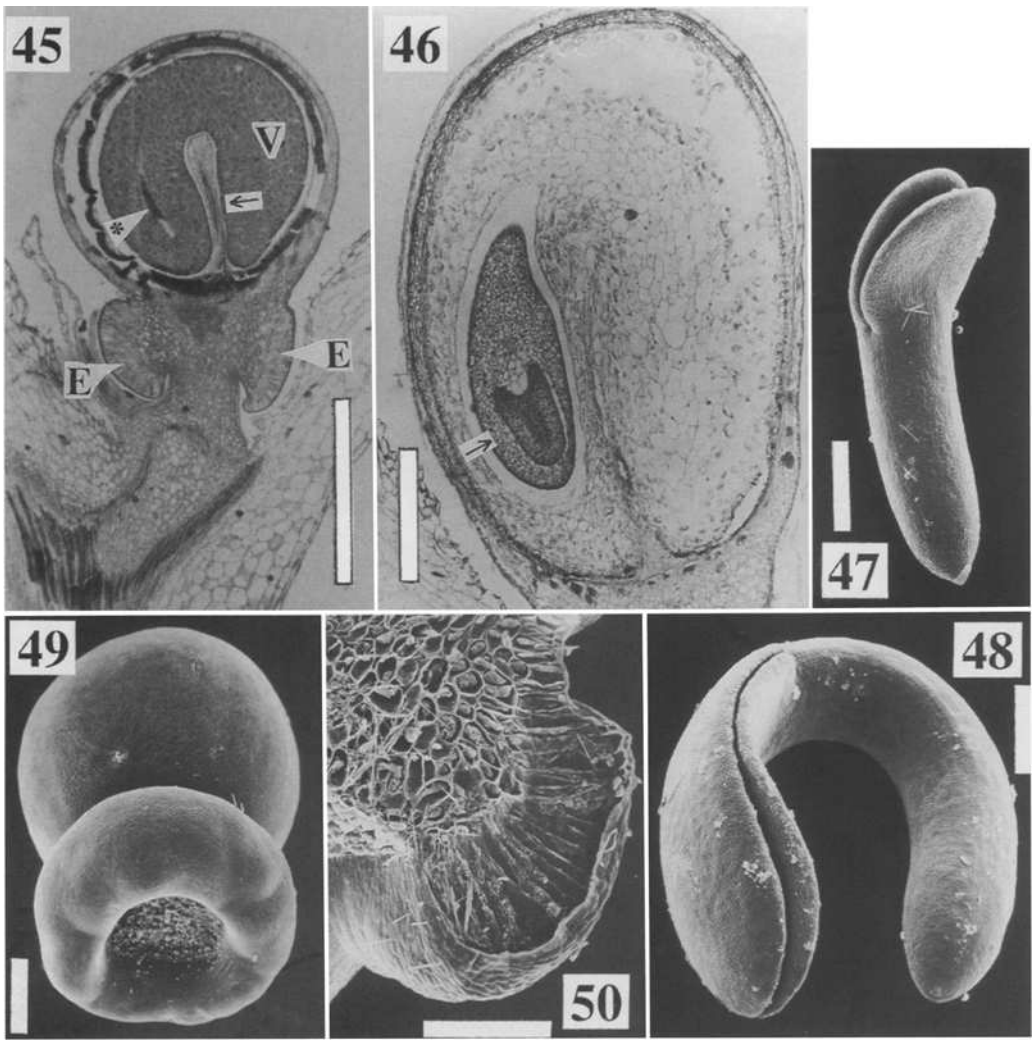


Figs. 34–38. *Theligonum cynocrambe*. Early development of female flowers. – Fig. 34. Early development of a female unit in leaf axil, seen from the side of the subtending leaf (L*, removed). First formed flower (♀1) of female unit (cyme) surrounded by two prophylls (I/I′), one stipule (SI*, removed) on the abaxial side, and another stipule (SI) on the adaxial side. – Fig. 35. Older female unit with central flower (♀1, with initial perianth), seen from the side of the subtending leaf (L*, removed). I/I′, two prophylls; SI, stipule belonging to the prophyllar pair. ♀2, position of next younger female flower (hidden). Note upper male flower (♂) belonging to another node. – Fig. 36. Slightly older developmental stage, central flower (♀1) with perianth rim. Abbreviations as above. – Fig. 37. Abaxial view of female unit. First formed flower (♀1) with initial three-lobed corolla and initial inferior ovary (already asymmetrically swollen). Younger lateral flower visible (♀2). Note interpetiolar adaxial stipule (SiI). Other abbreviations as above. – Fig. 38. Female flower (♀1) with displacement of the dimerous corolla to the adaxial side due to unequal growth of the inferior ovary. L*, subtending leaf (removed); M, position of mother shoot axis. – Bars: Figs. 34–36, 50 μm; Fig. 37, 100 μm; Fig. 38, 150 μm



Figs. 39–44. *Theligonum cynocrambe*. Female flowers at anthesis. – Fig. 39. Flower (♀1) at anthesis, with ovary (O), basilateral perianth (P) and long protruding stigma (G). ♀2, next younger flower bud in axil of prophyll (I). M, sectioned axis of mother shoot. – Fig. 40. Another flower at anthesis, with clearly bilobed perianth tube. Abbreviations as above. – Fig. 41. Distal portion of trilobed perianth tube (one lobe hidden). Note papillae on stigma. – Fig. 42. Lateral view of ovary at anthesis, with ovary wall partially removed in order to see the solitary campylotropous ovule arising from a basal placental bulge (arrow) that later on will form the septum. – Fig. 43. Transverse section through the flower base showing the ovule (V) and septum (arrow) dividing the ovary into two loculi. – Fig. 44. Longisection through young ovary with basal septum (arrow), one curved ovule (V) and elaiosome (E) at the flower base. – Bars: Figs. 39–40, 200 μm; Figs. 41–43, 100 μm; Fig. 44, 300 μm

developmental difference between the first flower (♀1 in Figs. 34–39) and the successive ones (♀2 in Figs. 37, 39). The terminal flower (♀1) arises centrally between the two prophylls, prior to the formation of an additional flower bud (♀2) in the axil of one of the two prophylls (Figs. 34, 37). Very often the smaller flowers do not reach maturity. Each female unit starts with the formation of a transversal pair of prophylls combined with one interpetiolar (= interfoliar) stipule on the



Figs. 45–50. *Theligonum cynocrambe*. Development of fruit and embryo. – Fig. 45. Longisection of very young fruit with curved ovule (V), basal septum (arrow), and region with small embryo (*). Note the well-developed elaiosome (E), showing radially elongated secretory cells. – Fig. 46. Longisection of young fruit showing still straight embryo (arrow), surrounded by endosperm. – Figs. 47, 48. Slightly and strongly curved embryo, with two cotyledons, of an immature and a mature fruit, respectively. – Fig. 49. Mature nut-like fruit, with ringlike elaiosome surrounding the insertion area of the globular one-seeded fruit. – Fig. 50. Longitudinal section of the elaiosome region, showing the elongated secretory cells. – Bars: Fig. 45, 1 mm; Figs. 46, 48, 300 μm; Fig. 47, 100 μm; Fig. 49, 500 μm; Fig. 50, 200 μm

adaxial side and two free and often minute stipules on the abaxial side (Figs. 36–37).

The female flower starts its development with the formation of a hemispherical primordium on which a ring primordium develops as the initial perianth tube (Figs. 34–36). No rudiments of a second (= calyx) perianth whorl are observable. Then

the inferior ovary becomes visible as a slightly asymmetrical inflated area by a constriction below the initial corolla tube (Fig. 37). In later developmental stages the corolla shows two or three lobes (Figs. 37, 38, 40). A single style pushes through the perianth tube and grows into a functional stigma covered with short papillae. The length of the style is variable (Figs. 39–41). Due to asymmetrical ovary growth the perianth and style position shifts downwards on the adaxial ovary side, leading to an almost gynobasic attachment of the style surrounded by the tubular perianth (Figs. 39–40; BALICKA-IWANOWSKA 1897: 364, fig. 8; WUNDERLICH 1971: 368; CRONQUIST 1981).

Developmental morphology of fruits and seeds. After anthesis the perianth and style of female flowers are shed, leaving a scar which is rapidly hidden by the inflation of the globular fruits. The nut-like fruits were often called drupaceous although the fleshy mesocarp is very thin. There are a few (two or three) parenchymatous layers and thick endocarp with brachysclereids (KAPIL & RAO 1966; and own observations, unpubl.).

There is only one basal and campylotropous ovule in the seemingly single locule of *Theligonum* (Figs. 42, 45). Similar to other *Rubiaceae* the reduction of the septum is accompanied by a stronger curvature of the single remaining ovule, which becomes campylotropous. This was also observed by WUNDERLICH (1971: 353): "Allem Anschein nach ist das Wachstum der Plazenta stark gehemmt, so dass die Samenanlage früh kampylotrop wird." After anthesis and fertilization the ovule becomes strongly curved leading to a seemingly globular seed with a basal incision that is occupied by a basal septum of the ovary. This septum divides the lower half of the ovary into two incomplete locules (see arrows in Figs. 42–45). Thus, the *Theligonum* ovary is basically bilocular as typical for most *Rubiaceae* with only one curved ovule that overtops and hides the basal septum.

During seed development the young embryo inside the curved ovule (mainly endosperm) starts with a nearly straight form finally becoming horseshoe-like with two cotyledons on one side and the radicular pole on the other side (Figs. 46–48). Ripe nut-like fruits show a prominent brown elaiosome that arises as a ringlike outgrowth from the insertion area of the inferior ovary after anthesis (E in Figs. 44–45). The elaiosome may be evenly ringlike or slightly lobed (Fig. 49). It consists of radially elongated cells that are rich of mucilage and oil serving as food for ants (Fig. 50; SERNANDER 1906, ULBRICH 1934).

Discussion

Rubiaceous affinity/palynology

The rubiaceous affinity of *Theligonum* was overlooked until 1971 (WUNDERLICH 1971; ROBBRECHT 1982, 1988, 1993) although the Russian botanist NENYUKOV already anticipated this view in 1939 (according to CRONQUIST 1981: 1001). ROBBRECHT (1993: 27) rightly refers to a "systematic camouflage". Based on palynological data, PRAGLOWSKI (1973), and NOWICKE & SKVARLA (1979) again questioned the inclusion of *Theligonum* into the *Rubiaceae*. According to them *Theligonum* should be placed in the *Caryophyllidae* (see also KAPIL & MOHANA RAO

1966). Pollen of the 4-8-zonoporate type is not found in other *Rubiaceae* although WUNDERLICH (1971) and PRAGLOWSKI (1973) described some palynological features that are shared by *Theligonum* and some *Rubiaceae*, especially *Anthospermeae* (also *Spermacoceae*, *Rubieae*): three-nuclear pollen, zonal arrangement of apertures, size and type of pori, tenuinexinous (compare also with ROBBRECHT 1982). The exine surface of *Theligonum* is almost the same as in *Paederia* subg. *Lecontea* (IGERSHEIM 1991). A table given by WUNDERLICH (1971) summarizes a number of similarities between *Theligonum* and other *Rubiaceae*.

Theligonum is either included in the *Rubiaceae*, subfam. *Rubioideae*, tribe *Theligoneae* (WUNDERLICH 1971; PUFF 1982, 1986; ROBBRECHT 1988, 1993; THORNE 1992) or placed in a small satellite family *Theligonaceae* in the *Gentianales* (MABRY & al. 1975, CRONQUIST 1981, DAHLGREN 1989, THEISEN & BARTHLOTT 1994). There are several morphological, anatomical and embryological arguments in favor of the *Rubiaceae* as next relatives of *Theligonum* (WUNDERLICH 1971): e.g., colleters, raphids, interpetiolar (interfoliar) stipules in the basal decussate region. In addition there is phytochemical, ultrastructural and molecular evidence supporting a rubiaceous affinity of *Theligonum*, excluding it from the subclasses *Caryophyllidae* and *Rosidae* (especially from *Haloragaceae*) (KOOIMAN 1971, MABRY & al. 1975, BREMER & al. 1995, NATALI & al. 1995, BREMER 1996, MANEN & NATALI 1996).

According to WUNDERLICH (1971) and PUFF (1982, 1986) the rubiaceous tribes *Anthospermeae* and *Paederieae* may be regarded as next related to *Theligonum* (= tribe *Theligoneae*, ROBBRECHT 1988, 1993). These three tribes may have 'common roots' (PUFF 1982, 1986: 519). *Theligonum* plants produce the foetid odour characteristic of many *Anthospermeae* and *Paederieae*, and the compounds asperuloside and galiumglucoside are also present (KOOIMAN 1971, PUFF 1982). The geographical distribution of *Theligonum* overlaps with that of several genera of *Paederieae*: Canary Islands, the Mediterranean area and east to Japan (ULBRICH 1934, PUFF 1982). Chloroplast DNA analysis data seem to support *Paederieae* such as *Putoria* besides *Rubieae* and *Didymeae* as close relatives of *Theligonum* (MANEN & al. 1994, and pers.comm.; NATALI & al. 1995, 1996; MANEN & NATALI 1996). BREMER & al. (1995) and BREMER (1996) also place *Theligonum* closer to the *Rubieae* and *Paederieae* than to the *Anthospermeae*. However, PUFF (1986, and pers. comm.) points to the strong similarity between *Theligonum* and the wind-pollinated *Anthospermeae*.

Features of *Theligonum* that are also found in other *Rubiaceae*

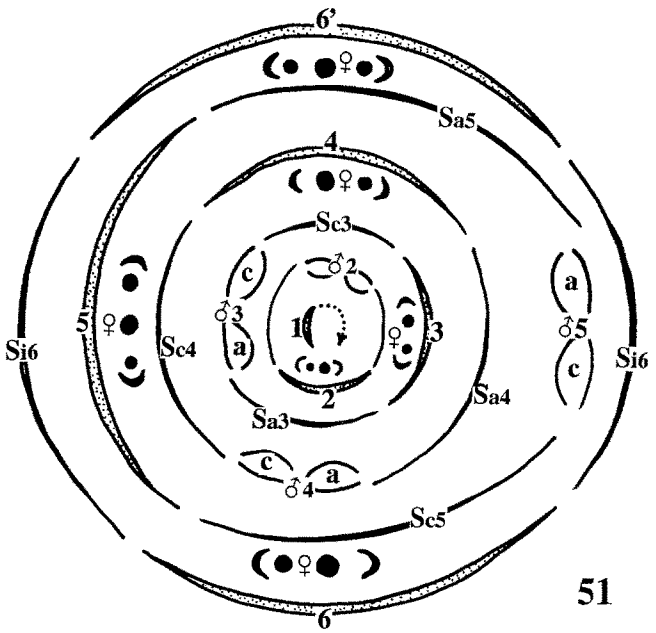
Anisophylly and 1/4 spiral phyllotaxis. The spiral phyllotaxis of *Theligonum* is traditionally explained by extreme anisophylly of a decussate system as typical for most *Rubiaceae*, with the (+) leaves of the basically decussate pattern arranged along a helix whereas all (−) leaves are suppressed (IRMISCH 1856; SCHNEIDER 1914; ROBBRECHT 1988, 1993). Anisophylly, i.e. the unequal development of the two leaves of an opposite pair, is clearly visible on the lateral branches of *Theligonum* and is very common in *Rubiaceae*, including *Anthospermeae*, as summarized by PUFF (1986: 519), and ROBBRECHT (1988: 48). The second (−) leaf of a pair may be

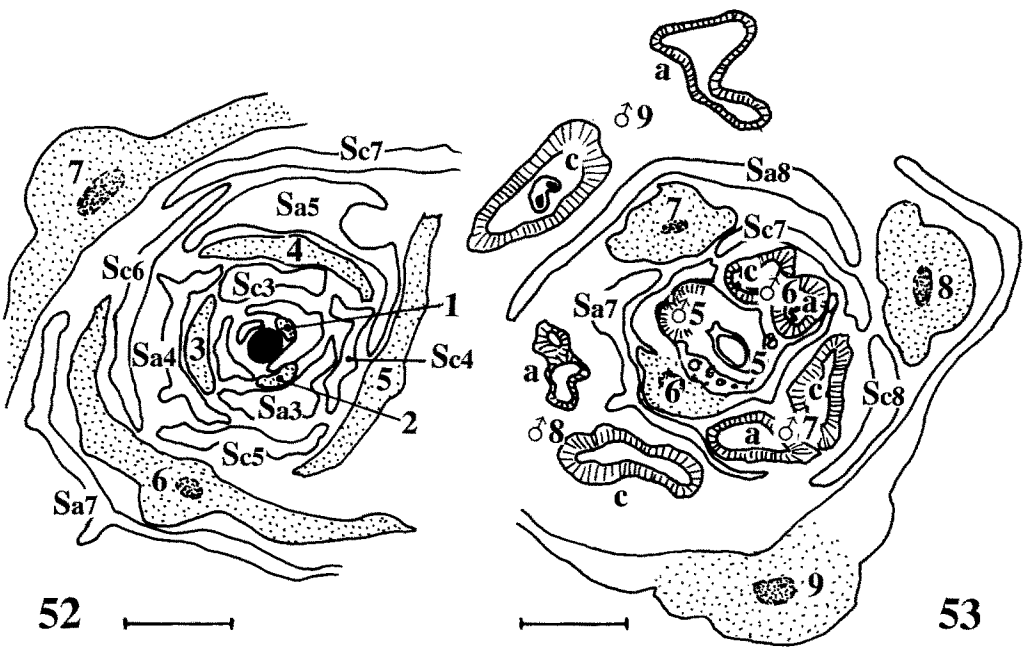
totally suppressed in other *Rubiaceae* such as *Pseudosabicea sthenula* (ROBBRECHT 1988: fig. 11 C), *Argostemma humilis* (SCHUMANN 1891: 22, fig. 8V). Because of the suppression of the (−) leaf in *Theligonum* the male flower pairs (rarely triplets) regularly arise without a subtending leaf, i.e. in an extra-axillary position. In *Theligonum cynocrambe* SCHNEIDER (1914: fig. 8) observed a shoot where two nodes again were provided with leaf pairs although the more proximal nodes showed 1/4 spiral phyllotaxis with one leaf per node. Anisophylly and anisoclady are spiral in many *Rubiaceae* (e.g., *Plocama pendula* of the tribe *Paederieae*, RUTISHAUSER 1981: 68–69, fig. 14 a–g; for other examples see SCHOUTE 1938).

The spiral phyllotaxis with a divergence of 90–100° is consistent with the suppression hypothesis of one leaf per pair. There is, however, another possibility to interpret the 1/4 spiral phyllotaxis in *Theligonum*. It might be interpreted as a Lucas system (with divergence d approximating 99.5°): Figs. 2, 52, 53 and show the mean divergence closer to 99° than to 90°! Also LOISEAU (1969) mentioned in *Theligonum* divergence angles d > 90°. The cross-sections of *Theligonum* shoot apices (Figs. 52, 53) reveal divergence angles around 100°. Both Lucas systems (d=99.5°) and Fibonacci systems (d=137.5°) are spiral phyllotactic patterns related to the Golden angle (RUTISHAUSER 1981, 1982, 1997): In contrast to the widespread Fibonacci systems typical Lucas systems are restricted to plant taxa with narrow insertion angles (i) of the leaves ($i \leq 70°$): certain conifers, *Crassulaceae* (*Sedum* spp.), *Euphorbiaceae* (e.g., *Euphorbia myrsinites*). *Theligonum*, therefore, would be an exceptional Lucas system because of leaf insertion angles up to 300° when the leaf and the two associated stipules are taken as one developmental unit (Figs. 6, 11, 52).

Complete reduction of the calyx. As already explained in the introduction, the simple perianth of *Theligonum* is best homologized with the corolla of other *Rubiaceae* (e.g., tribes *Anthospermeae*, *Paederieae*, *Rubieae*, *Spermacoceae*), also having valvate aestivation (WUNDERLICH 1971; ROBBRECHT 1988: 82). The corolla development of *Theligonum* corresponds to the early sympetaly sensu ERBAR (1991) observed in some other *Rubiaceae* (ERBAR & LEINS 1996). There is a similar depression surrounded by a low girdle. Early floral ontogeny of *Rubiaceae* without calyx (e.g., *Galium*, *Cruciata*) resembles *Theligonum* to a great extent (PÖTTER & KLOPFER 1987, ERBAR 1991, ERBAR & LEINS 1996). This coincides with the tendency of calyx reduction in other *Rubiaceae*: *Coffea* with only a short calyx rim, *Spermacoce* spp. with minute lobes, *Phyllis nobla* (tribe *Anthospermeae*) with a strongly reduced calyx (MENDOZA-HEUER 1977), *Neogaillonia* (tribe *Paederieae*; ROBBRECHT 1988: 74) and *Galium* (PÖTTER & KLOPFER 1987) with calyx totally absent.

Dimerous to trimerous flowers. Flowers in *Rubiaceae* are mostly pentamerous. However, a trend to tetramerous flowers can be observed in almost every tribe (ROBBRECHT 1988). Trimerous flowers are exceptional. Besides *Theligonum* trimerous flowers are found in rubiaceous genera such as *Alibertia*, *Asperula*, *Corynanthe*, *Mitchella*, *Opercularia*, *Pouchetia* (see ROBBRECHT 1988: 73, ERBAR & LEINS 1996: Figs. 6–7). Dimerous flowers in *Rubiaceae* seem to be restricted to *Theligonum*. A simple explanation for the derivation of dimery is by the lateral fusion (interprimordial growth) of petals leading to a reduction of sectors in the flower. Indeed, the adaxial petal in dimerous flowers is occasionally larger and enclosing more stamens than the abaxial one (Figs. 23, 24).





Figs. 51–53. *Theligonum cynocrambe*. Branching pattern, position of the male and female flowers, 1/4 spiral phyllotaxis. Note that the leaves are labeled with Arabic numerals according to their age from shoot tip to base, with youngest leaf numbered 1. – Fig. 51. Diagrammatic scheme of young plant (similar to Fig. 2) showing switch from uppermost leaf pair (6/6′) to spirally arranged leaves (5, 4, 3, 2, 1). Ontogenetical helix drawn in clockwise direction. Uppermost leaf pair with interpetiolar stipules (Si6). Each of the

Dimorphic flowers with lack of the opposite sex (or nearly so). Dimorphic flowers occur in various *Rubiaceae* (WUNDERLICH 1971: 368). Monoecy or even dioecy can be found abundantly in this family. Sexual flower morphs of unequal size (male flowers with larger corolla than the female ones) are relatively frequent in the tribe *Anthospermeae* (WUNDERLICH 1971: fig. 6, MENDOZA-HEUER 1977, FLORENCE 1986, PUFF 1986): e.g., *Anthospermum*, *Coprosma*, *Nertera*, *Phyllis*. In some genera of *Anthospermeae* (*Anthospermum*, *Carpacoce*, *Galopina*, *Nenax*, *Phyllis*) the corolla lobes are curved backwards as is typical for the male flowers of *Theligonum* (WUNDERLICH 1971, MENDOZA-HEUER 1977, PUFF 1986: fig. 33 g).

Male and female flowers of *Theligonum* seem to lack any rudiment of the opposite sex. We did not observe rudimentary stamens inside the corolla tube of female *Theligonum* flowers. Occasionally the male flowers of *Theligonum* possess a globular base (Fig. 30) that may be homologous to a rudimentary inferior ovary. This globular base is similar in size and shape with the rudimentary ovary of male flowers in *Nenax* spp. (PUFF 1982, 1986: fig. 33 e–g). In *Nenax* and other *Anthospermeae* with floral dimorphism the opposite sex may be observable just as a rudiment during floral development. Only exceptionally (e.g., in *Anthospermum*) male flowers lack any gynoecial rudiment (WUNDERLICH 1971: 369; PUFF 1986).

Polyandry and basal fusion of filaments in male flowers. There are only two rubiaceous genera (*Theligonum*, *Coprosma*) with an increased stamen number if *Dialypetalanthus* is not regarded as a member of the *Rubiaceae* (FLORENCE 1986, PIESSCHAERT 1995). In *Theligonum* the stamen number may considerably exceed the number of corolla lobes whereas *Coprosma* (especially *C. nephelephila*) shows a slight increase of stamen number (5–12) in flowers with five or six corolla lobes (FLORENCE 1986, ROBBRECHT 1993). In the *Theligonum cynocrambe* material studied we did not observe male flowers with more than 19 stamens although up to 30 stamens per flower are mentioned in the literature (SCHNEIDER 1914, WUNDERLICH 1971, CRONQUIST 1981, ROBBRECHT 1988). Additional developmental studies are needed in order to find out if there are other rubiaceous genera (tribes *Anthospermeae*, *Paederieae*) besides *Coprosma* having at least slightly more stamens than corolla lobes. In the monotypic Canarian genus *Plocama* (tribe *Paederieae*) the male flowers show five to seven stamens but this seems to be always correlated with an identical number of five to seven corolla lobes (MENDOZA-HEUER 1987; PUFF 1982: table 2, 1991).

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spirally arranged leaves with two lateral stipules, the anodic stipules (Sa5,Sa4,..) slightly more vigorous than the cathodic ones (Sc5,Sc4,..). Female units (♀) in axils of leaves (6, 5, 4,..), each unit cymose, consisting of c. 2–3 female flowers. Male units extraaxillary, opposite the single leaves (5, 4,..), consisting of slightly unequal male flowers: anodic flower (♂a) adjacent to anodic stipule of same node, and cathodic flower (♂c) adjacent to cathodic stipule. – Figs. 52–53. Two cross-sections of an apical shoot bud. All symbols as mentioned in Fig. 51. – Fig. 52. Level of the shoot apical meristem (black area). – Fig. 53. Insertion level of leaf 5. All leaves (dotted areas) are arranged along a spiral with divergence of c. 100°. The stipules (white) of each leaf are unequal, with the anodic stipule (Sa) slightly bigger than the cathodic one (Sc). Male flowers (♂, hatched areas) in pairs, without subtending leaf, opposite to the leaf. – Bars: Figs. 52, 53, 500 µm. – Fig. 51 redrawn from EICHLER (1878), Figs. 52 and 53 redrawn from LOISEAU (1969)

The position of androecial primordia in flowers with few stamens of *Theligonum* (two or three depending on the number of petals!) alternate with the positions of the petals as usual for *Rubiaceae* and other *Asteridae*. Especially male flowers with a dimerous corolla may show an elliptical rim (common girdling primordium) in early development. This rim is then divided up into several stamen primordia nearly simultaneously following the curvature of the petals. The loss of a calyx in *Theligonum* (linked with variable floral symmetry) may have induced a chaotic increase of the stamens (cf. ENDRESS 1994). There is mostly one stamen in alternipetalous position. The observation of BALICKA-IWANOWSKA (1897) and SCHNEIDER (1914) who described epipetalous primordia is therefore only partially true.

We only exceptionally found fascicle formation among the stamens of *Theligonum* (groups of stamens with fused filament bases as partial monadelphy) as hypothesized by WUNDERLICH (1971). However, we do not exclude an increased ratio of partial monadelphy in other *Theligonum cynocrambe* populations because the initial girdling primordium (Figs. 14–15) may give rise to an androecial tube or stamen fascicles (partial monadelphy) quite easily. An androecial tube was found in other *Rubiaceae* such as *Exostema*, *Chiococca* (*Portlandia* group, subfam. *Antirheoideae*) as well as *Strumpfia* by BREMER & STRUWE (1992) and IGERSHEIM (1993, also pers. comm.). In these genera it is necessary to study early androecium development in order to know if there is also an initial common girdling primordium similar to that found in *Theligonum*.

Trend towards a unilocular ovary. Most authors, such as BALICKA-IWANOWSKA (1897), WUNDERLICH (1971) and ROBBRECHT (1988: 90) described *Theligonum* with an unilocular ovary that does not show any trace of the second locule. In *Rubiaceae* with a normally bicarpellate ovary there are only very few taxa with a total reduction of the second locule. In *Otiophora* and *Calanda* the unilocular ovary still possesses a trace of the second carpel (ROBBRECHT 1988: 90).

In the post-anthesis ovary of *Theligonum cynocrambe* the lower third is provided with a septum that shows a delayed development as compared to the single ovule arising from its center (Figs. 42–44). The attachment of the ovule is situated at the base of the septal ridge. Thus, *Theligonum* has an ovary that is bilocular in the lower region.

WUNDERLICH (1971: 353, fig. 3g–h) already saw this septum. She called it placental tissue. Thus, as already BALICKA-IWANOWSKA (1897), WUNDERLICH (1971) concluded that *Theligonum* does not show any trace of the second carpel (see ROBBRECHT 1988: 90 for a similar statement). WUNDERLICH interpreted the septum as a special formation of the basal placenta that may be derived from the transversal zone ("Querzone") of the only carpel. We agree with WUNDERLICH only to some extent. In various *Rubiaceae* with a reduced ovule number the ovules arise from the center of the upper rim of the incomplete septum (ROBBRECHT 1988). DE BLOCK (1995) also described the reduction of one of the two ovules of *Rutidea* (Pavetteae) and the filling-up of the whole cavity by the seed, which literally invades the sterile locule. As in *Theligonum* the septum is incomplete, but there is an apical residue of the septum separate from the basal part. Thus, the only ovule of the *Theligonum* ovary arises at the very same site except that this septum shows a somewhat delayed development.

Curved (campylotropous) ovules with insertion along their concave side seem to be present in various *Rubiaceae* (Figs. 45–46, ROBBRECHT 1988, ROBBRECHT & al. 1991). Especially those *Rubiaceae* members with a reduced ovule number (or even an uniovular ovary) often show seeds with coiled endosperm and horseshoe-like embryo (A. IGERSHEIM, pers. comm.). Some *Rubiaceae*, however, show a unilocular ovary with a single anatropous ovule (including straight endosperm and embryo), as mentioned by PUFF (1986: *Carpacoce* spp.) and ROBBRECHT (1988: *Calanda*, *Otiophora*).

Anemophily. Most *Rubiaceae* are entomophilous (WUNDERLICH 1971: 369). However, in the tribes *Anthospermeae* and *Paederieae* there is a tendency towards anemophily with pendulous stamens, elongated filaments, and versatile anthers (e.g., in *Anthospermeae*: *Nertera*, *Phyllis*), as stated by MENDOZA-HEUER (1972, 1977, 1987), PUFF (1986: 519). Thus, the anemophily of *Theligonum* is not exceptional in *Rubiaceae* (ROBBRECHT 1988).

Conclusions and outlook

This paper provides a better knowledge of the developmental morphology of *Theligonum* (tribe *Theligoneae*) as compared to other *Rubiaceae*. *Theligonum* seems less exceptional than thought until now. Some of its features are observable as evolutionary trends already in other tribes of subfam. *Rubioideae*. For example, 6–7-zonoporate pollen grains also occur in some *Borreria* spp. (tribe *Spermacoceae*; PIRE 1996). Male flowers of *Theligonum* occasionally are provided with a base that may be interpreted as a rudimentary ovary. Female flowers show a bilocular ovary with an incomplete septum that is overarched by the only ovule. We agree with ROBBRECHT'S (1993) conclusion that "*Theligonum* is a very advanced evolutionary line within *Rubiaceae*". It is justified to speak of a leakiness of the "Bauplan" in *Rubiaceae* especially when we focus on the polyandry of male flowers in *Theligonum* with a developmental pattern not found or very rare in other *Asteridae*. There are other features such as the elaiosome, the twisting of dehisced stamens, papillate filaments, gynobasic style associated with an inferior ovary that are not (yet) known from other *Rubiaceae*. Still needed is a cladistic analysis including anatomical and morphological characters besides molecular data in order to find the closest relatives of *Theligonum* within the *Rubiaceae* (BREMER & STRUWE 1992, MANEN & al. 1994, BREMER & al. 1995, BREMER 1996, NATALI & al. 1995, MANEN & NATALI 1996). This analysis is in progress.

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